Project Report: Evolution in Microbe−Based Ecosystems: Desert Springs as Analogues for the Early Development and Stabilization of Ecological Systems

Lead Team:	***Arizona State University***
Project Title:	***Evolution in Microbe−Based Ecosystems: Desert Springs as Analogues for the Early Development and Stabilization of Ecological Systems***
Project Investigators:	***James Elser, Jack Farmer***

Project Progress

Overview. During the four expeditions of 2001−2002 (our second year of funding), we carried out extensive integrated sampling and experimental studies at a number of field study sites in the Cuatro Cienegas Basin, Central Mexico. In addition, we processed samples from previous expeditions and prepared data for presentations at several scientific meetings and for publication. Key results for each project component are briefly described below.

Stoichiometry. We continued to characterize nutrient supply conditions in various habitats in the basin, concentrating our efforts in the Churince drainage and in the Rio Mezquites. Field sampling indicated that PO_4 is in short supply (relative to inorganic N and other potential chemical limiting factors) and indeed algal mats and stromatolite biomass in the basin appear to have extremely high C:P and N:P ratios. Consistent with this potentially poor food quality, a 3−week P−fertilization experiment was successful in lowering the C:P ratio of microbial biomass in Rio Mezquites stromatolites. This lowering of microbial C:P stimulated snail P content and ribonucleic acid (RNA):DNA ratio, indicating that snail growth in this system is limited by stoichiometric food quality (low dietary P−content).

Snail Morphometry (Tang/Rooparnine). We completed intensive quantitative morphometric analysis of Mexipyrgus from various localities in the basin. These results indicate an extraordinary degree of morphometric differentiation in this taxon. Preliminary findings from this work have been submitted to *Astrobiology*. Additional samples are being processed and we are now preparing DNA for analysis so that we can determine if this morphometric diversity is associated with genetic differentiation.

Pupfish (Dowling). We have completed foundational work for this study by

performing basin−level, population genetic analyses of mitochondrial and nuclear DNA in *Cyprinodon bifasciatus* and *C. atrorus* (Carson and Dowling 2002, In Preparation), and phylogenetic analysis of *Cyprinodon* mitochondrial DNA (Echelle et al 2001, Submitted). Currently we are assessing whether physiological differences between *C. bifasciatus* and *C. atrorus* have important influences on local adaptation of introgressed *Cyprinodon* within the environmental gradient between these species.

Cyanobacteria (Garcia−Pichel). We completed (and published) a study related to the buoyancy regulation of calcite−producing colonial cyanobacteria ("waterwarts") in Posa Escobedo. In addition, we characterized cyanobacteria communitry structure along longitudinal transects in Rio Mezquites using molecular techniques (denaturing gradient gel electrophoresis (DGGE)) and in the process developed a new procedure for DNA extraction from carbonate−dominated samples. We also completed detailed studies on metabolic processes, including calcification, in Rio Mezquites stromatolites using microelectrode techniques.

Archaea and Eubacteria (Souza, Eguiarte). Field samples from several dozen more localities were obtained from around the basin. We were successful in establishing >2500 cultures of Eubacteria in the laboratory as well as a variety of isolates of Archaea. Preliminary genetic analysis by Restriction Fragment Length Polymorphism (RFLP) indicates that all of the >2500 Eubacteria isolates are genetically distinct.

Stromatolite Morphogenesis and Microbial Taphonomy (Farmer). This year, we began studies of the microbial paleontology (taphonomy) and morphogenesis of oncolitic stromatolites (spherical stromatolites formed by rolling). Long−term *in situ* experiments were deployed at three sites in the Rio Mezquitas to determine the average accretion rate and transport history for oncoids. Samples were collected and fixed in the field. Characterization of these samples by scanning electron micrograph (SEM) and light microscopy showed that oncoids possess distinctly zoned communities consisting of a surficial assemblage of larger (>10 micron diameter) filamentous cyanobacteria and diatoms overlying a subsurface community (1−2 mm depth) dominated by finely−filamentous and coccoidal (**Modeling (Fagan, Odell).** Little additional progress in ecosystem modeling was made during the past year, and the subcontract with the University of Washington was terminated.

Highlights

- **“Multidisciplinary team continues studies in “living laboratory” of early evolution”:** During 2001−2002, a diverse team of limnologists, microbial ecologists, evolutionary biologists, and paleontologists extended its intensive study of unique desert springs in the ancient valley of Cuatro Cienegas, Mexico. There, by studying stromatolite colonies and other microbial forms similar to those that dominated life on early Earth, they hope to better understand the ecological and environmental forces that shaped and stabilized our planet's earliest food webs. Lead investigators include Prof. Jim Elser (ecological

stoichiometry; Biological Sciences, Arizona State University), Prof. Jack Farmer (stromatolite studies; Dept. of Geological Sciences, Arizona State University), Prof. Ferran Garcia Pichel (ecology of cyanobacteria and microbial mat studies; Dept. of Microbiology, Arizona State University), Prof. Tom Dowling and Evan Carson (fish ecology and evolution; Biological Sciences, Arizona State University), Dr. Carol Tang (paleontology and species diversity of snails; Cal Academy of Sciences), and Drs. Valeria Souza and Luis Eguiarte (microbial diversity; UNAM, Mexico City)

- **"Did a junk food diet limit the success of early animals in the Precambrian?":** By fertilizing living stromatolite−forming algae with inorganic PO_4,researchers working at Cuatro Cienegas, Mexico, have found evidence that low concentrations of mineral PO_4 in the diets of herbivorous snails may limit their growth and reproduction. If such conditions are analogous to those experienced on the early Earth, then one might hypothesize that such stoichiometric constraints may have contributed to the long delay in the appearance of complex animals (Metazoa) that now dominate our planet's food webs. The primary investigator in this work is Prof. Jim Elser (Dept of Biology, Arizona State University).

- **"Hyperdiversity in a desert Galapagos":** Quantitative studies of snail morphometry and microbial and fish genetic diversity indicate that the desert thermal springs at Cuatro Cienegas, Mexico, are a hotbed of biological differentiation. Morphometric analysis of one snail taxon in the basin, members of the genus *Mexipyrgus*, indicates that each habitat sampled so far in the basin contains its own morphometrically distinct form. Paralleling this diversity in the snail fauna, genetic analysis of more than 2500 separate isolates of aquatic bacteria in the basin indicates that each strain is genetically distinct from every other strain. High diversity is paralleled even in larger organisms, such as endemic desert pupfish, which show signs of sharp genetic differentiation across environmental gradients in local habitats. Primary investigators in this work are Drs. Carol Tang and Peter Rooparnine (snails; California Academy of Sciences), Prof. Tom Dowling and Mr. Evan Carson (pupfish, Arizona State University), and Drs. Valeria Souza and Luis Eguiarte (microbes; UNAM, Mexico City).

Roadmap Objectives

- ***Objective No. 3:*** Models for Life
- ***Objective No. 4:*** Genomic Clues to Evolution
- ***Objective No. 5:*** Linking Planetary Biological Evolution
- ***Objective No. 6:*** Microbial Ecology
- ***Objective No. 7:*** Extremes of Life
- ***Objective No. 14:*** Ecosystem Response to Rapid Environmental Change

Mission Involvement

Jack Farmer is Chair of the Mars Exploration Assessment Group (MEPAG), the primary community planning group for Mars exploration and the MEPAG Astrobiology Science Steering Group (SSG), which serves as a forum for astrobiological mission planning for Mars. Farmer is also Chair of the NAI Mars Focus Group, the forum within the NAI for the astrobiological exploration of Mars. Jim Elser and Carol Tang are members of MEPAG's Mars Pathways Science Steering Group, providing input to the Mars mission planning community regarding science−driven investigation pathways for the Mars Smart Lander in 2009, as well as for the decade beyond. They, along with Ferran Garcia−Pichel, are also members of MEPAG's Astrobiology SSG, which provides long−range planning recommendations for the astrobiological exploration of Mars.

Field Expeditions

Field Trip Name:	
Start Date: 02/01/2001	***End Date:*** 08/01/2001
Continent: North America	***Country:*** Mexico
State/Province: Coahuila	***Nearest City/Town:*** Cuatro Cienegas
Latitude: 26 deg 50 00 N	***Longitude:*** 101 deg 02 00 W
Name of site(cave, mine, e.g.): Rio Mezquitas and the Churince drainage network	***Keywords:*** limnology, genetics, food webs, diversity, stromatolites, cyanobacteria, paleontology
Description of Work: Field sampling and experimentation associated with stromatolite, microbial, and metazoan studies in various habitats and field study sites in the Cuatro Cienegas Basin.	
Members Involved:	